



Effects of load mass carried in a backpack upon respiratory muscle fatigue.

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TITLE:

Effects of load mass carried in a backpack upon respiratory muscle fatigue

RUNNING TITLE:

Load carriage and the respiratory muscles

ABSTRACT

Purpose: The purpose of this study was to investigate whether loads carried in a backpack, with a load mass ranging from 0 to 20 kg, causes respiratory muscle fatigue.

Methods: Eight males performed four randomised load carriage (LC) trials comprising 60 min walking at $6.5 \text{ km}\cdot\text{h}^{-1}$ wearing a backpack of either 0 (LC₀), 10 (LC₁₀), 15 (LC₁₅) or 20 kg (LC₂₀). Inspiratory ($P_{I_{\max}}$) and expiratory ($P_{E_{\max}}$) mouth pressures were assessed prior to and immediately following each trial. Pulmonary gas exchange, heart rate, blood lactate and glucose concentration and perceptual responses were recorded during the first and final 60 s of each trial.

Results: Group mean $P_{I_{\max}}$ and $P_{E_{\max}}$ were unchanged following 60 min load carriage in all conditions ($p>0.05$). There was an increase over time in pulmonary gas exchange, heart rate and perceptions of effort relative to baseline measures during each trial ($p<0.05$) with changes not different between trials ($p>0.05$).

Conclusions: These findings indicate that sub-maximal walking with no load or carrying 10, 15 or 20 kg in a backpack for up to 60 min does not cause respiratory muscle fatigue despite causing an increase in physiological, metabolic and perceptual parameters.

Key Words

Respiratory muscle fatigue, chest wall loading, chest wall restriction, load carriage, exercise performance

Introduction

Carrying equipment is an essential requirement in certain occupational and recreational settings (Birrell & Haslam, 2010; Swain, Ringleb, Naik, & Butowicz, 2011). Typically loads are carried in a backpack located upon the posterior thorax supported by shoulder straps and/or a hip belt. This position is the most effective and practical placement for an external load (Knapik, Reynolds, & Harman, 2004). When carrying a load, a positive relationship is observed between load mass and oxygen uptake ($\dot{V}O_2$), heart rate and pulmonary ventilation with loads up to 30 kg (Borghols, Dresen, & Hollander, 1978) becoming non-linear with heavier loads up to 65 kg ($>70\% \dot{V}O_{2\max}$) (Christie & Scott, 2005; Patton, Kaszuba, Mello, & Reynolds, 1991).

The positioning of backpacks upon the thorax presents a challenge to normal breathing mechanics and predisposes the respiratory system as a limiting factor of exercise tolerance (Dominelli, Sheel, & Foster, 2012; Faghy & Brown, 2014b). Wearing a heavy backpack provides an inspiratory volume limitation of the thorax, forcing the respiratory muscles outside of the most efficient portion of their length-tension curve (Brown & McConnell, 2012; Dominelli et al., 2012). External loads carried in this way also cause autonomous changes in posture such as an increased anterior trunk displacement which increase lumbosacral (L5/S1) forces and spinal stiffness (Goh, Thambyah, & Bose, 1998). Changes in the amplitude of lumbosacral forces that perturb the spine are related to diaphragm activation even during slow and rapid movements of the upper limbs per-se (Hodges & Gandevia, 2000). Therefore diaphragm activation and the activation of other respiratory muscles attached to the spine and thorax will be magnified further during exercise with load carriage.

A positive linear relationship has been demonstrated between respiratory muscle work and backpack load mass during brief (5 min) walking exercise when carrying loads of 15, 25 or 35 kg at $4.0 \text{ km}\cdot\text{h}^{-1}$ (Dominelli et al., 2012) which specifically occurs to preserve pulmonary function and spinal stiffness. Indirect assessment of the external intercostals and the sternocleidomastoid inspiratory muscles using EMG during 60 min exercise at $8 \text{ km}\cdot\text{h}^{-1}$ with a 15 kg backpack demonstrated a reduction in their mean power frequency (Nadiv et al., 2012), a proxy for respiratory muscle fatigue. In addition, we have repeatedly demonstrated that performing load carriage exercise at $6.5 \text{ km}\cdot\text{h}^{-1}$ while wearing a 25 kg for up to 60 min consistently reduces the volitional inspiratory and expiratory mouth pressures; a global measure of the force generating capacity of these muscles (Faghy & Brown, 2014a, 2014b, 2015) which is not present during the same exercise without a load (Faghy & Brown, 2014b).

To date therefore, the effect of carrying loads less than 25kg upon volitional respiratory muscle fatigue, measured directly by the transient change (pre- vs post-exercise) in mouth pressures remains unknown. This is important as lighter loads have greater relevance in some occupational and recreational groups. Therefore, the aim of this study was to examine the effects of load carriage mass (0, 10, 15 and 20 kg) carried in a backpack system upon respiratory muscle fatigue, physiological variables and perceptual responses during constant velocity sub-maximal load carriage.

Methods

Participants

Following ethics approval from the University ethics committee, 8 healthy, non-smoking and physically active males, familiar with load carriage through regular recreational load carriage activities (e.g. hiking, outdoor activities and cadets) provided written informed consent to participate in the study (age: 20.9 ± 0.8 yr; stature: 1.81 ± 0.09 m; body mass: 75.1 ± 11.6 kg; body mass index [BMI]: 23 ± 2.7 kg/m²). Throughout the study participants did not engage in any strenuous exercise on the day preceding and the day of an exercise test. Each participant completed a 24 h diet record prior to their first exercise trial, which was then repeated prior to all subsequent trials. Participants abstained from alcohol and caffeine in the 24 h prior to testing and arrived at the laboratory 2 h post-prandial.

Experimental Design

Participants attended a briefing session where the experimental design was outlined in detail. Following this, participants completed one preliminary trial and four experimental trials. The order of the experimental trials was randomised for each participant using a Latin square and each trial was separated by a minimum of one week. This approach to between-day trials has been shown previously by our group to maximise reliability in load carriage assessment (Faghy & Brown, 2014a).

Preliminary Trial

Participant's body mass (Salter 145BKDR, HoMedics, Kent, UK) and stature (Seca 217, USA) were measured and BMI calculated. Participants were familiarised with all testing equipment and protocols and completed baseline peak expiratory flow and maximal inspiratory ($P_{I_{max}}$) and expiratory pressure ($P_{E_{max}}$) measurements. All manoeuvres were performed in

accordance with published guidelines (American Thoracic Society & European Respiratory Society, 2002) and expressed relative to predicted values using published equations (Wilson, Cooke, Edwards, & Spiro, 1984). P_{Imax} and P_{Emax} were measured as an index of global inspiratory and expiratory muscle strength, respectively, using a hand-held mouth pressure meter fitted with a flanged mouthpiece (MicroRPM, Micro Medical, Kent, UK). The mouthpiece assembly incorporated a 1 mm orifice to prevent glottic closure and minimise the contribution of the buccal muscles during efforts. All manoeuvres were performed standing with inspiratory efforts initiated from residual volume and expiratory efforts performed from total lung capacity, and sustained for at least 3 s. A minimum of 3 and maximum of 8 manoeuvres were performed every 30 s, and the maximum value of 3 measures that varied by <5% was used for subsequent analysis (American Thoracic Society & European Respiratory Society, 2002). Although high levels of participant motivation is required for these efforts, when sufficient time is given to familiarise participants as was achieved here, these measures are highly reproducible before and after 60 min load carriage exercise (Faghy & Brown, 2014a; Romer & McConnell, 2004).

Participants were then familiarised with the backpack loads (Web Tex, Bedford, UK) and wearing the heaviest load (20 kg) performed a full habituation of the experimental trial (see below). An absolute load mass was selected in favour of a load relative to body mass to directly reflect current training and operational requirements of the Armed Forces and Emergency Services (Rayson, Holliman, & Belyavin, 2000). During all load carriage trials, the load mass was evenly distributed within the central compartment of the backpack and worn in accordance with the manufactures guidelines and previous work from our laboratory (Faghy & Brown, 2014a, 2014b). The backpack incorporated two shoulder straps and a waist strap which were

adjusted individually and recorded to the nearest mm for subsequent trials. All experimental trials were performed on a motorised treadmill (Desmo, Woodway, Germany).

Experimental trials

Measures of pulmonary function and respiratory muscle strength (see above, *preliminary trial*) were collected at baseline (i.e., prior to exercise) and post exercise. Physiological variables were collected during the first and last 60 s of exercise as detailed below. Following this, participants walked for 60 min, 0% gradient and 6.5 km·h⁻¹ carrying a backpack (hereon referred to as Load Carriage: LC) with no load (backpack mass = 1 kg, LC₀) or whilst carrying 10 kg (LC₁₀), 15 kg (LC₁₅) or 20 kg (LC₂₀).

Heart rate was measured using short-range telemetry (HR; Polar T31, Kempele, Finland) and averaged over 60 s. Expired pulmonary gases were assessed using Douglas bags (Cranlea and Co, Birmingham, UK). Expired gas samples were analysed for F_EO₂ and F_ECO₂ using a gas analyser (Hitech Instruments, GIR250, Cranlea, Birmingham, UK) and volume using a dry gas meter (Harvard Apparatus, Cranlea, Birmingham, UK). Blood lactate ([lac⁻]_B, Accu-Check, Safe T-Pro, Birmingham, UK) and glucose concentrations ([glu]_B, Accutrend blood glucose, Birmingham, UK) were assessed from fingertip capillary blood samples. Ratings of whole body perceived exertion (RPE) were measured using the Borg 6-20 scale (Borg, 1982). Perceptions of effort were separated for leg (RPE_{legs}) and breathing (RPE_{breathing}) discomfort using a visual analogue scale: where 0 = no exertion and 10 = maximal exertion (Faghy & Brown, 2015).

Statistical Analysis

Statistical analysis was performed using SPSS for Windows (SPSS, Chicago, IL, USA). A one-way repeated measures ANOVA was used to confirm that there were no differences between

variables at baseline between trials. Two-way repeated measures ANOVA was used to examine changes in each of the dependent variables over time and between conditions. Where significant main effects for each trial (LC₀, LC₁₀, LC₁₅ and LC₂₀) and time (baseline/start vs. end exercise) or interaction effects (trial x time) were revealed, paired sample t-tests were used to determine differences at specific time points with a Bonferroni correction applied for multiple comparisons. A priori α was set at 0.05, all results are presented as mean \pm SD and effect size (ES) reported for pairwise comparisons.

Results

There were no differences between load carriage conditions at baseline for any variables (Table 1; $p>0.05$).

Respiratory muscle pressures

Baseline values of $P_{I_{\max}}$ and $P_{E_{\max}}$ were not different between trials ($p>0.05$) and were within normal limits relative to predicted values. Data for each trial are reported in Table 1 and pooled here to provide an overview. Pooled baseline $P_{I_{\max}}$ and $P_{E_{\max}}$ were 108 ± 13 cmH₂O (92 ± 12 % of predicted) and 145 ± 18 cmH₂O (90 ± 11 % of predicted) respectively. Relative to baseline, there was no change ($p>0.05$) in $P_{I_{\max}}$ and $P_{E_{\max}}$ following LC₀ (no load) and when wearing a backpack with a load mass of 10, 15 or 20 kg ($p>0.05$) (see Table 1).

Physiological and perceptual responses

The physiological and perceptual responses measured in the first and last 60 s of load carriage are shown in Table 1. There was no main effect of time for RER, $[\text{lac}^-]_{\text{B}}$, and $[\text{glu}]_{\text{B}}$, ($p>0.05$), showing that these parameters did not change during the 60 min exercise trial. $\dot{V}\text{CO}_2$, RPE_{legs} and $\text{RPE}_{\text{breathing}}$ showed a main effect for time ($p<0.05$) but no interaction effect ($p>0.05$) indicating that the change in these parameters over time was similar between load carriage conditions. HR, \dot{V}_{E} , $\dot{V}\text{O}_2$ and RPE showed a main effect for time ($p<0.05$) and a significant interaction effect ($p<0.05$). Post-hoc analysis revealed that end-exercise HR was higher in LC₂₀ compared to LC₀ (mean difference: 28 ± 12 beats·min⁻¹; $p<0.01$, ES= 0.19). \dot{V}_{E} was greater during the final 60 s of load carriage during LC₂₀ when compared to the same time point in LC₀ (mean increase: 13.4 ± 3.5 L·min⁻¹, $p<0.001$, ES= 0.16). Similar changes were also observed for $\dot{V}\text{O}_2$, $\dot{V}\text{CO}_2$ and accordingly, RER remained unchanged. $[\text{lac}^-]_{\text{B}}$ and $[\text{glu}]_{\text{B}}$ values were not different between trials at baseline and also following exercise ($p>0.05$).

****TABLE ONE HERE****

Discussion

The purpose of this study was to investigate the effects of load mass worn in a backpack system (four trials: backpack with no load, 10, 15 and 20 kg load) upon respiratory muscle fatigue, physiological and perceptual responses. In particular, the transient change in respiratory and expiratory muscle pressures before vs. after 60 min of treadmill walking at 6.5 km·h⁻¹ was investigated. The novel finding was that load mass carried in a backpack system had no effect upon respiratory muscle pressures (i.e., respiratory muscle fatigue) in any trial. Our findings demonstrate increased physiological responses with increased load mass up to 20 kg that is carried within a backpack; however, despite this the volitional force generating capacity of the respiratory muscle remains unaffected during constant velocity load carriage.

Respiratory muscle pressures

A reduction in respiratory muscle pressures (prior to vs. post immediately post-exercise) is indicative of respiratory muscle fatigue (Romer & McConnell, 2004). This study demonstrated that respiratory muscle fatigue was not present following 60 min load carriage with a load mass ranging from 0 to 20 kg, i.e. there was no change in respiratory muscle pressures pre vs post exercise. Recent evidence with participants of similar anthropometric and aerobic fitness to this study demonstrated, on two separate occasions, the absence of respiratory muscle fatigue when no load was carried (Faghy & Brown, 2014a, 2014b). In contrast these studies, that employed identical experimental design, demonstrated a reduction in both $P_{I_{max}}$ and $P_{E_{max}}$ from baseline (~11% and 13% respectively) following 60 min load carriage at 6.5 km·h⁻¹ while wearing a 25 kg backpack. Our current findings are however in contrast to others (Nadiv et al., 2012) who demonstrated increased EMG activity (a proxy of skeletal muscle fatigue) of the sternocleidomastoid (94%) and external intercostal (49%) during 15 kg load carriage exercise at 8 km·h⁻¹ for 60 mins. This method has however been highly criticised for its use as a surrogate

measure of skeletal muscle fatigue (Sheel & Romer, 2012). Thus despite the lower load mass yet faster walking pace, which in combination may contribute to respiratory muscle fatigue with a lower backpack load mass, caution is warranted when interpreting the conclusions presented by Nadiv et al. (2012) as only three of their eight participants completed the loaded exercise protocol. Therefore, we suggest that a threshold load mass may exist (between 20 kg to 25 kg) during 60 min load carriage exercise at $6.5 \text{ km}\cdot\text{h}^{-1}$ beyond which respiratory muscle fatigue ensues. Therefore, due to differences in walking speed, load mass and methods of determining respiratory muscle fatigue in the present and past studies it is not possible to establish whether this suggested load carriage threshold of respiratory muscle fatigue at $6.5 \text{ km}\cdot\text{h}^{-1}$ is applicable at other walking speeds with different exercise intensity.

The position of the load, location of the shoulder straps and hip belt alters breathing mechanics through restriction of the anterior regions of the thorax, imposing a volume limitation of the chest wall (Dominelli et al., 2012). To date the only study that has investigated the effects of chest wall restriction upon respiratory muscle and pulmonary function, using inelastic chest wall strapping (i.e., with no load; (Tomczak, Guenette, Reid, McKenzie, & Sheel, 2011). Here it was demonstrated that low intensity cycling exercise ($\sim 40\% \dot{V}O_{2\text{peak}}$) impairs lung volumes and flow rates, and using bilateral phrenic nerve stimulation to evoke and intrathoracic pressure balloon catheters to measure twitch force, causes significant diaphragm fatigue (Tomczak et al., 2011). Therefore the contribution of load to this condition is likely to exacerbate diaphragm fatigue however; the effect of thoracic loads upon evoked twitch force of the respiratory muscles remains unknown during occupational tasks and warrants further investigation.

The work of breathing is linearly related to backpack load mass, and relative to unloaded exercise, places the respiratory muscles on an inefficient portion of their pressure-volume

(length-tension) curve lowering compliance (Dominelli et al., 2012). This reduces respiratory muscle efficiency during prolonged exercise (>60 min) and with sufficient load (>20 kg), causes inspiratory muscle fatigue (Faghy & Brown, 2014a, 2014b). With an external load mass up to 20 kg, we suggest that the shift in breathing mechanics remains within the high compliance zone of the pressure-volume curve and therefore does not affect the force output of the respiratory muscles. However, when the load mass exceeds 20 to 25 kg, a significant shift in operational lung volumes may occur, such that the force generating capacity of the respiratory muscles is impaired and respiratory muscle fatigue ensues (Faghy & Brown, 2014a, 2014b).

Despite showing consistent findings in our data of no respiratory muscle fatigue within all load mass trials (see Table 1), whether a threshold exists in all individuals beyond this study however is unknown. It is known that respiratory muscle strength is determined by a number of factors including sex, lung volume, whole body strength, age and body mass (Wilson et al 1984) as well as the relative contribution of the chest wall and the diaphragm to inspiratory force generation (Brown, Johnson, & Sharpe, 2014). Load carriage performance is also determined by a number of key parameters including familiarity with load carriage, sex, whole body strength, body mass, and aerobic capacity (i.e. $\dot{V}O_{2peak}$; (Haisman, 1988). Therefore, the load mass-mediated threshold of respiratory muscle fatigue proposed here may also be affected by any individual and/or combination of these parameters outlined above and future work should investigate these interactions.

Physiological and perceptual responses

Increased heart rate, $\dot{V}O_2$ and perceptual responses with increasing load mass are in agreement with previous literature (Beekley, Alt, Buckley, Duffey, & Crowder, 2007; Borghols et al., 1978; Christie & Scott, 2005). It is surprising that increased physiological strain with

increasing load mass was not mirrored by greater respiratory muscle fatigue. The onset of respiratory muscle fatigue is dependent upon respiratory muscle work history and systemic disruptions caused by locomotor muscle physiology (Babcock et al., 1995). In the present study, the magnitude of these was presumably not great enough, alone or in combination, to induce respiratory muscle fatigue. A limitation of the present study however was the lack of measurement of torso muscle activity and/or force producing capability and to date no studies have directly compared these changes with increasing load mass (i.e. 0-20 kg). Holewijn (1990) observed a linear increase in m. trapezius activity when walking with increasing load (0, 5.4 and 10.4 kg). We have also demonstrated previously that carrying a 25 kg load for 120 min at 6.5 km·h⁻¹ causes fatigue of the shoulder flexors and trunk extensors and flexors (Blacker, Fallowfield, Bilzon, & Willems, 2010). Therefore our data indicates there is no relationship between the increase in cardiovascular strain and energy cost with load mass and the threshold limit of respiratory muscle fatigue, which deserves further exploration.

Methodological limitations

We employed global volitional measures of respiratory muscle force in this study, which do not directly reflect the force output of the diaphragm or indeed any other specific respiratory muscle. Rather, they reflect the volitional force output of all respiratory muscles working in synergy as occurs during dynamic exercise. However, despite this we have demonstrated previously that $P_{I\max}$ and volitional maximal trans-diaphragmatic pressure ($P_{di\max}$) are correlated (Brown, Johnson, & Sharpe, 2014), therefore we suggest that our measures of $\Delta P_{I\max}$ provide a true measure of inspiratory muscle force and a useful surrogate of diaphragm and chest wall function. In addition, although non-volitional measures of muscle force are preferred, due to technical limitations volitional measures were employed. Consequently, in line with previous work (Faghy & Brown, 2014a, 2014b), we spent much time ensuring

familiarisation with this technique and strove to maximise motivation throughout all efforts to optimise measurement reliability (Faghy and Brown 2014a). We therefore are confident that any potential effects of reduced subject motivation and/or effort were minimised. Finally, unlike previous studies we did not quantify the work of breathing, breathing mechanics (Dominelli et al., 2012; Tomczak et al., 2011) or respiratory muscle activation (Nadiv et al., 2012) and future studies should seek to address this.

Practical application

Respiratory muscle fatigue has important consequences for occupational and recreational activities where thoracic load carriage using a backpack systems is employed. Consequently, occupational performance is likely to be impaired when carrying loads ≥ 20 to 25 kg for ≥ 60 min, in addition to the effects of the increased metabolic (Borghols et al., 1978) and neuromuscular demands (Blacker et al., 2010). Respiratory muscle training (RMT) is a simple technique that targets both the strength and endurance characteristics of the respiratory musculature (Romer & McConnell, 2003). We recently demonstrated for the first time that 6 wk RMT increases the threshold for inspiratory and expiratory muscle fatigue following 60 min load carriage, attenuates cardiovascular strain, perceptions of effort and improves subsequent 2.4 km time trial performance when carrying a 25 kg thoracic load in a backpack system (Faghy & Brown, 2015). Since a load mass of less than 20 kg does not result in respiratory muscle fatigue, our data suggest that these training methods may not be appropriate for the exercise intensity and duration used in this study and hence may not improve subsequent 2.4 km time trial performance. However, RMT has been shown to attenuate perceptual and cardiovascular responses (variables which are elevated during exercise carrying backpack loads <25 kg) during exercise where respiratory muscle fatigue is not observed (Brown, Sharpe, & Johnson, 2012). Therefore, under these conditions (i.e., low intensity exercise with backpack load mass <25 kg), RMT may provide an ergogenic aid although this is yet to be determined.

Conclusion

Sub-maximal walking with no load or carrying 10, 15 and 20 kg in a backpack does not cause respiratory muscle fatigue despite causing an increase in physiological, metabolic and perceptual parameters. We propose a threshold load exists (20 to 25 kg) during 60 min exercise at 6.5 km·h⁻¹ beyond which respiratory muscle fatigue ensues and future research should investigate the factors contributing to this proposed threshold.

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Integrity of Research and Reporting

Ethical Standards

All experimental procedures and methods of assessment used in this study were ethically approved by the host universities ethics committee and conform to the laws of the United Kingdom.

Conflicts of interest:

No conflicts of interest for each of the authors.

Funding:

None

Table 1. Respiratory muscle pressure at baseline and post exercise; pulmonary ventilation, gas exchange, cardiovascular and perceptual responses during the first and final 60 s of exercise.

	LC ₀		LC ₁₀		LC ₁₅		LC ₂₀	
	Baseline	Post-exercise	Baseline	Post-exercise	Baseline	Post-exercise	Baseline	Post-exercise
$P_{I\max}$ (cmH ₂ O)	95 ± 20	93 ± 16	98 ± 17	95 ± 18	96 ± 20	94 ± 19	97 ± 19	95 ± 15
$P_{E\max}$ (cmH ₂ O)	127 ± 12	128 ± 18	133 ± 24	132 ± 23	129 ± 21	127 ± 21	131 ± 18	132 ± 22
	First 60 s	Final 60 s	First 60 s	Final 60 s	First 60 s	Final 60 s	First 60 s	Final 60 s
\dot{V}_E (L·min ⁻¹)	31.2 ± 5.3	33.5 ± 5.5	34.6 ± 7.2	37.4 ± 3.3 ^E	35.9 ± 5.5	38.3 ± 5.3 ^{AE}	36.5 ± 4.4	45.8 ± 6.2 ^{*+A}
$\dot{V}O_2$ (L·min ⁻¹)	1.5 ± 0.3	1.6 ± 0.4 ^E	1.5 ± 0.3	1.7 ± 0.2 ^A	1.5 ± 0.3	1.8 ± 0.3 ^A	1.7 ± 0.2	2.1 ± 0.4 ^{AB}
$\dot{V}CO_2$ (L·min ⁻¹)	1.0 ± 0.2	1.0 ± 0.5 ^{DE}	1.3 ± 0.2	1.4 ± 0.1 ^{AB}	1.3 ± 0.3	1.5 ± 0.2 ^{AB}	1.5 ± 0.3	1.7 ± 0.4 ^{AB}
RER	0.8 ± 0.1	0.7 ± 0.9	0.9 ± 0.1	0.8 ± 0.2	0.8 ± 0.1	0.9 ± 0.2	0.9 ± 0.1	0.8 ± 0.1
[lac ⁻] _B (mmol·l ⁻¹)	1.2 ± 0.5	1.3 ± 0.9	1.2 ± 0.5	1.2 ± 0.5	1.0 ± 0.2	1.2 ± 0.8	1.0 ± 0.2	1.4 ± 0.8
[glu] _B (mmol·l ⁻¹)	5.0 ± 0.9	4.6 ± 0.7	4.9 ± 1.4	4.6 ± 0.8	4.9 ± 0.6	4.0 ± 1.0	4.7 ± 0.5	4.3 ± 0.9
HR (beats·min ⁻¹)	113 ± 11	119 ± 17 ^{AE}	120 ± 18	123 ± 19 ^{ABE}	122 ± 17	130 ± 20 ^{ABE}	132 ± 20 ^B	143 ± 25 ^{*+A}
RPE (AU)	6 ± 0	9 ± 3 ^{AE}	6 ± 0	9 ± 2 ^{AE}	6 ± 0	10 ± 4 ^{AE}	6 ± 0	13 ± 3 ^{AC^U}
RPE _{legs} (AU)	0 ± 0	1 ± 1 ^E	0 ± 0	2 ± 2 ^{AE}	1 ± 1	3 ± 3 ^A	0 ± 0	4 ± 3 ^{ABC}
RPE _{breathing} (AU)	0 ± 0	1 ± 1	0 ± 0	1 ± 1	0 ± 0	2 ± 2 ^A	0 ± 0	3 ± 2 ^{A^U}

Maximum inspiratory pressure ($P_{I\max}$), Maximum expiratory pressure ($P_{E\max}$), Minute ventilation (\dot{V}_E), oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), Respiratory exchange ratio (RER), Blood Lactate [lac⁻]_B, Blood glucose [glu]_B, heart rate (HR), arbitrary units (AU), Rating of perceived exertion (RPE), Rating of perceived exertion specific to the legs (RPE_{legs}), Rating of perceived exertion specific to breathing (RPE_{breathing}). ^A = different from start of exercise, ^B = different from LC₀, ^C = different from LC₁₀, ^D = different from LC₁₅, ^E = different from LC₂₀, ^{*} = different between all trials. ⁺ effect size <0.2 between LC₀ and LC₂₀, ^U effect size <0.2 between LC₁₅ and LC₂₀.